PATENT
Attorney Docket No.3.0-008

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

Menozzi et al.

Serial No.:09/192,579 Group Art Unit: 1645

Filed: November 17, 1998 Examiner: R. Swartz

For: IDENTIFICATION AND CLONING OF A MYCOBACTERIAL ANTIGEN CORRESPONDING TO A HEPARIN-BINDING HAEMAGGLUTININ

DECLARATION PURSUANT TO 37 C.F.R. § 1.132

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

I, Franco Dante Menozzi, do hereby state and declare the following:

1. I am a co-inventor of the subject matter disclosed and claimed in the above-captioned application. I received a Doctorate in Biological Sciences from the University of Mons-Hainaut, Belgium in 1987 and am currently working at the Institut Pasteur of Lille in Lille, France. I have been a member of the American Society for Microbiology from 1991 to 2003. My Curriculum Vitae is attached as Exhibit 1.

2. I have reviewed the latest U.S. Official Action mailed December 11, 2003. It appears to me that in this Official Action the Examiner deems that the above-captioned patent application is anticipated by Menozzi et al. *Abstracts of the General Meeting of the ASM* 95(0):193, Abstract B-159. I have also reviewed the current claims in this application which recite the specific heparin binding site of HBHA and which encompass 30 to 50 amino acids of the C-terminal portion of SEQ ID No. 19 (Figure 10 in the application). It is my opinion that there was insufficient information

set forth in this Abstract for a skilled scientist to identify the heparin binding site of HBHA from the disclosure therein for the following reasons.

3. From the observations disclosed in Abstract B-159 it is my opinion that it would be impossible for the skilled scientist to map or even localize the heparin-binding site within the HBHA protein. Such a mapping required the cloning of the HBHA-encoding gene, as well as the development of biological assays based on the capability of native or recombinant HBHA fragments to interact with heparin or related sulphated polysaccharides. These assays are not described nor mentioned in Abstract B-159 and as a consequence no pertinent information or investigative strategy was given to the skilled artisan that could lead to the mapping of the HBHA heparin-binding site.

4. Even if a scientist could in fact clone and express the HBHA gene at that epoch, the identification of the heparin binding site could only be achieved by producing the recombinant form of HBHA in *E. coli* and with further analysis of the expression product. As set forth on pages 20 to 23 of the specification, several additional experiments were performed which led us to identify the heparin binding site in HBHA. These additional analysis included lysing the HBHA produced in *E. coli* and analysis of the apparent molecular weight protein, which was recombinantly produced, further chromagraphic analysis on heparin-sepharose of a clarified sonicate of *E. coli* producing the recombinant HBHA, analysis of recombinant HBHA by immobilised heparin matrix chromatography, microsequencing the 26 kDA and 25 kDA degradation products of the complete 27 kDA recombinant protein and analyzing the results obtained. None of this information is disclosed in Abstract B-159.

5. In addition mapping of the HBHA heparin-binding site required inventive ingenuity since any conclusion concerning such a mapping could not be drawn without knowing that:

(1) HBHA migrates in an aberrant fashion in a SDS-polyacrylamide gel electrophoretic analysis; and

(2) the adhesion is prone to proteolysis.

These two important features of HBHA have been demonstrated following recombinant expression of HBHA in *E. coli* and microsequencing of the peptides eluted from the heparin-sepharose column, respectively.

6. Furthermore, it should be noted that the experiments performed in paragraph 4 above, it was essential to use the monoclonal antibody 3921E4 in the analysis. This antibody is not described in Abstract B-159. Nor is the other monoclonal antibody 4057D2, used in the experimental expression section of the above-captioned patent application described in Abstract B-159. These monoclonal antibodies were crucial to the analysis of the results. For instance, the fact that the protein was present in *Mycobacterium tuberculosis* and it was surface associated could only be ascertained by using the antibodies 4057D2 and 3921E4, which were not described in Abstract B-159. Moreover, the fact that this isolated protein was called HBHA was known using the 4057 D2 antibody. Using the same 4057 D2 antibody it was known that this isolated 28 kDA protein was different from the antigen 85 complex. Therefore, there is no information in Abstract B-159 that would allow a skilled scientist to study structurally or functionally HBHA using known specific immunological reagents.

7. Therefore, in conclusion it is my belief that a scientist faced with the disclosure in Abstract B-159 could not, without additional information and guidance as given in the present specification, obtain the amino acid sequences of the heparin binding site of HBHA.

8. I further declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

1st March 2004

Date

Franco Dante Menozzi